From the simulation results, we more directly extract the rate at which supercoiling in DNA is relaxed by nicking endonucleases. We also determine the dependence of the relaxation timescales on the tension applied to the DNA molecule.

1176-Pos Board B127
Charge Redistribution in Excited State Lumichrome and Lumazine
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Flavins (vitamin B2 and its derivatives) are important blue light sensors and photoredox cofactors. They are also crucial for many proteins that do ground state redox chemistry. Lumichrome (Lc) and lumazine (Lm) are photodegradation products of flavins. Lumazine has been identified as a photoantenna for the cryB protein from Rh. sph., which provides photoregulation of photosynthesis in that cyanobacterium (J. Biol. Chem. 2014, 289:19659-19669). Other studies (J. Biol. Chem. 2009, 284:13068-13076) suggest that archaeal dodecin, a riboflavin-binding protein, may reduce Rbf concentrations photoselectively by an ultrafast electron transfer reaction to produce LC in a manner that preserves cellular integrity. We have used Stark spectroscopy to measure the electronic charge redistribution accompanying light absorption for the lowest optically accessible singlet transitions. Difference dipole moments and changes in the polarizability of the molecules were estimated. These results are discussed in relation to the proteins that incorporate them.

1177-Pos Board B128
Complexes of G-Quadruplex DNA with Drug Like Molecules
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Structural information on the complexes of drug like molecules with quadruplex DNAs can aid the development of therapeutics and research tools that selectively target specific quadruplex DNAs. Screening can identify candidate molecules that require additional evaluation. An enhanced hydroxyl radical cleavage protocol is demonstrated that can efficiently provide structural information on the complexes of the candidate molecules with quadruplex DNA. NMR methods have been used to offer additional structural information about the complexes as well as validate the results of the hydroxyl radical approach. This multistep protocol has been demonstrated on complexes of the quadruplex formed by the thrombin binding aptamer (TBA) and promoter region of c-kit. The hydroxyl radical results indicate that NSC 176319, Cain's quinolinium that was found by screening, exhibits selective binding to the two TT loops. The NMR results are consistent with selective disruption of the hydrogen bonding between T4 and T13 as well as unstacking of these residues from the bottom quartet. The preliminary result indicates that the NSC 176319 also binds to the loops of the quadruplex formed by the promoter region of c-kit. NSC 176319 is used as a hit and the structurally similar molecules are being screened to find other candidate molecules. Thus, the combination of screening, hydroxyl radical footprinting and NMR can find new molecules that selectively bind to quadruplex DNAs as well as provide structural information about their complexes.

1178-Pos Board B129
Determinants of Self Aggregation of H-Tel
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Guanine rich oligodeoxyribonucleotides (ODNs) can form non-canonical DNA structures known as G-quadruplexes. These are four stranded structures stabilized by monovalent sodium and potassium cations. The topologies of folded G-quadruplexes are highly polymorphic. H-Tel, an ODN with four consecutive repeats of the human telomeric sequence, [d(AGGGTTAGGGTTAGGGTAGGG)], can assume different monomolecular G-quadruplex topologies depending on the type of cation present in solution. In vivo a large portion of cellular volume is occupied by macromolecules; which can also affect the topology adopted by folded H-Tel. At high concentrations the DNA itself can also contribute to the crowding conditions, which may be relevant to the behaviour of DNA in the cell. Our previous work demonstrated that at high concentrations of the guanine rich sequences, the monomolecular G-quadruplexes formed by H-Tel self-associate to form higher order structures. Such aggregates display a circular dichroism spectrum similar to that of an all-parallel structure. We are investigating the energetics and mechanism of the interaction between the individual folded H-Tel monomers. Using H-Tel and H-Tel derivative ODNs with modified loop sequences, we are studying the contributions of the loops interactions to the self-association of monomolecular G-quadruplexes folded by H-Tel. The structural change from a G-quadruplex monomer to an aggregate is studied as a function of time and ODN concentrations. Specifically, we used circular dichroism spectroscopy (CD), UV spectroscopy, and gel electrophoresis to study the thermodynamics and kinetics of the folding and self-aggregation of these ODNs. We are also studying the energetics of the folding of the cytosine-rich complementary strand with the same methods. The thermodynamics of the concomitant folding of the G-rich and C-rich strands may play a role in the behaviour of these sequences in the cell.

1179-Pos Board B130
The DNA Dynamics Near Nanopores at Sub-Millisecond and Sub-Micrometer Levels by the Ultraviolet Light Spot
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Phenomenon of deoxyribonucleic acid (DNA) translocation through a nanopore is a significant interest in the field of biophysics. In the condition of applied voltage, coiled DNA molecules in the electrolyte approach to the mouth of a nanopore and thread into a nanopore by uncoiling its structure. Then DNA molecules recoil and diffuse outward from a nanopore after DNA translocation. Because DNA molecules uncoil to thread into a nanopore and recoil after DNA translocation, DNA dynamics near a nanopore strongly correlate with DNA translocation. Here, we propose an optical method for investigating DNA dynamics near nanopores at sub-millisecond and sub-micrometer levels by using ultraviolet light and silicon nanopores. Silicon nanopores, which have high refractive index and extinction coefficient at ultraviolet light, induces the light spot, whose size is 100nm, upon nanopores due to low transmitted light condition. By threading into nanopores, DAPI-stained DNA molecules pass through the ultraviolet light spot and emit fluorescence. We use the fluorescence intensity trace as the information of DNA translocation through nanopores. As the results of our measurement, the fluorescence intensity traces were in accordance with previous investigation of the voltage dependence of DNA electrophoresis mobility. Furthermore, our analytical results of fluorescence intensity traces showed the correlation between DNA conformations during DNA translocation through nanopores, which are unfolded or folded DNA translocation, and DNA dynamics after DNA translocation through nanopores, which reveal the usefulness of our optical method for investigating DNA dynamics near nanopores.

1180-Pos Board B131
Hydroxymethylation of DNA alters Nucleosomal Properties in vitro
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Hydroxymethylation of DNA at the C5 position of cytosine (5hmC) is an important epigenetic modification, which has an established connection with transcriptional regulation. 5hmC is an intermediary of the demethylation pathway and is considered to be a transcriptionally active mark. The mechanistic role of 5hmC in regulating transcription, however, remains poorly understood. We sought to understand the molecular role of 5hmC by studying its impact on DNA and nucleosomal properties. This was assessed through measurements of nucleosome stability, compactness and the free energy of DNA-histone binding. The 5hmC-induced changes were compared with another well-documented modification of DNA, i.e., methylation at the C5 position of cytosine (5mC). Our results show that the hydroxymethylation of DNA increases the affinity of DNA for the histone octamer, thus favoring nucleosome formation. Hydroxymethylation was also found to lower the stability of formed DNA-histone complexes in a salt induced dissociation experiment. We were able to trace the origin of this effect to the weakened affinity of the 5hmC DNA for the H2A-H2B dimers. The conformation of formed nucleosomes was altered slightly by the presence of DNA modifications. This was mainly reflected in a change in the end-to-end distance of the nucleosomal DNA, involving up to 4 base pairs. Our results suggest that hydroxymethylation of DNA plays an opposing role in nucleosomal dynamics, when compared to methylation. Hydroxymethylation of DNA can possibly lead to a more transcriptionally active state, which is consistent with its role in the demethylation pathway.

1181-Pos Board B132
DNA Diffusion is Dependent on Ionic Strength
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Capillary electrophoresis can be employed to measure the translational diffusion of ionic molecules using very small volumes of very dilute solutions. Diffusion is observed by increases in peak width when the analyte remains in the capillary for variable periods of time in the absence of an electric field. We have made this technique more efficient by repeatedly reversing the direction of the electric field, thus allowing the ionic molecules to pass the detection window multiple times after a single injection.
We have applied this technique to measure the translational diffusion coefficients of double-stranded DNA molecules ranging in size from 71 to 960 base pairs, injected into buffers of different ionic strengths. Surprisingly, we find that the diffusion coefficients decrease with increasing ionic strength;